# Cytoskeletal Protein Dynamics

**839-Pos Board B594**

**Microtubule Organization in Living Cells Studied with a New Single Filament Tracking Routine**

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The cytoskeleton is involved in numerous cellular processes such as migration, division and contraction and provides the tracks for transport driven by molecular motors. Therefore, the quantification of the mechanical behavior of cytoskeletal filaments is essential for understanding cell mechanics and organization. Previous works analyzed the motion and shape fluctuations of fluorescent microtubules and demonstrated that relevant mechanical properties of these biopolymers can be derived from this analysis. However, tracking individual fluorescent filaments in living cells is extremely complex due to the high and inhomogeneous background. We introduce a new tracking algorithm that allows recovering the coordinates of microtubule segments in living cells with 5-10nm precision. To illustrate potential applications of this algorithm, we studied the curvature distributions of fluorescent microtubules in living cells. By performing Fourier analysis of the microtubule shapes, we found that the curvatures followed a thermal-like distribution as previously reported with an effective persistence length of ~15um, significantly smaller than that measured in vitro. We also verified that either depolymerization of actin or intermediate filaments decreased the persistance length while expression of the microtubule-associated protein XTP increased this value. In addition, we recovered trajectories of microtubule segments in actin or intermediate filament depleted cells, and observed a significant increase of their motion with respect to untreated cells showing that these filaments contribute to overall organization of the microtubule network. Moreover, the analysis of trajectories of microtubule segments in untreated cells showed that these filaments presented slower but more directional motion in the cortex than in the perinuclear region and suggests that the tracking technique would allow mapping the microtubule dynamical organization in living cells. The tracking method could also be used to study the correlation between organelle motion and microtubule dynamics.

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**Probing Single Pair Protein-Protein Interaction in Live Cells by BiFC-PALM**

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It is essential to image dynamics of interacting protein pairs, but often comes across difficulties following a single pair in a crowded cell, due to the fluorescent background from other pairs nearby, in addition to that from non-paired proteins which are also fluorescently labeled. FRET is a powerful approach to study protein-protein interactions. Nonetheless, given the limited FRET efficiency, it is difficult to observe fast motion of interacting pairs in a short time scale. Here we present a new method named BiFC-PALM utilizing a split mEos3.2, to achieve super-resolution imaging as well as dynamics of single protein pairs in live cells. By fusing the mEos3.2 fragments to an interacting pair MreB-EF-Tu, we observed the detailed distribution of the interacting parts in Escherichia coli with a lateral resolution of ~25nm, and tracked the movement of single interacting pair with a 20ms time resolution. The molecule pair trajectories revealed two types of movement modes: one is relative immobile while the other is fast diffusing with diffusion coefficient 0.5um2/s. The two modes are further proved to be involved in cell wall synthesis and cell shape maintenance.
In summary, BiFC-PALM promises a valuable method to study protein-protein interaction with high specificity and spatial-temporal resolution in live cells.

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**NDC80 Microtubule Binding but not Cooperativity Decreases Proportionally to the Number of Phosphorylated Residues and Independently of their Positions in NDC80 Tail**

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Proper regulation of dynamic interactions between microtubules and chromosomal kinetochores is vitally important for accurate cell division, but little is known about how such regulation is achieved. Here, we used single molecule and quantitative fluorescence microscopy approaches to dissect microtubule binding of the multisubunit NDC80 complex, a core kinetochore microtubule-binding component. Human GFP-labeled NDC80 proteins were designed with up to nine phospho-mimetic aspartates, all located in the N-terminal tail of Hec1 subunit in a different combinations. Previous studies have suggested that one set of these phospho-residues controls primarily the affinity of NDC80 binding to microtubule, while the other set regulates binding cooperativity. We provide evidence, however, that phosphorylation of Hec1 tail has little impact on the cooperativity of NDC80 binding. In contrast, the microtubule binding affinity, as estimated from the residency time and diffusion coefficient of single NDC80 complexes, is highly sensitive to Hec1 tail phosphorylation. The binding energy decreases linearly at about 0.3 kBT per added phosphoresidue regardless of its specific position in Hec1 tail. To gain insight into such unusual regulatory behavior we have carried out molecular dynamics simulations of the disordered Hec1 tail in the context of microtubule-bound NDC80 complexes. These theoretical simulations revealed multiple conformations of the Hec1 tail bound to microtubule. Interestingly, the contact area between Hec1 tail and microtubule decreases proportionally to the number but not the locations of phospho-mimetic mutations in Hec1 tail. We propose that the disordered nature of NDC80 tail renders its microtubule binding insensitive to the exact location of phosphorylated residue, thereby enabling the gradual tuning of kinetochore-microtubule binding affinity during mitosis.

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**Multivalent Binding and Diffusion of Isolated Kinetochore Particles on Microtubule Lattice in Vitro**

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The kinetochore is a protein complex that connects spindle microtubules and centromere DNA. During mitosis, kinetochores establish correct bi-oriented attachment of spindle on sister chromatids, and mediate the segregation of sister chromatids in anaphase. Throughout this process, kinetochores remain attached on the plus ends of the microtubules, which undergo rapid depolymerization to effectively pull chromosomes apart. Moreover, kinetochores are able to exert force to the centromere DNA to overcome the viscous drag on the chromosome. How kinetochores harness the energy released from the microtubule depolymerization to do work is under active debate.
Recent advances in isolating kinetochore particles enable the possibility to test how kinetochores interact with microtubule in vitro. Using fluorescently labeled kinetochores purified from budding yeast, we are able to observe the dynamic binding, diffusion, pausing, and detachment events of single kinetochore particles on microtubule lattice. Consistent with the stoichiometry knowledge of kinetochore architecture that each kinetochore contains multiple microtubule binding units, the Ndc80 complex, we find that the resident time increases and the diffusion decreases as the number of binding unit increases. The kinetics observation can be explained with a biased-diffusion model, from which the binding and detachment rate, diffusion constant, and pausing rate are extracted. Our observation suggests that biased-diffusion is a plausible mechanism for kinetochore to interact with shrinking microtubule tip. We also show that the binding affinity of kinetochore with microtubule is regulated by the phosphorylation state and the presence of Dam1 complex, as means to establish correct microtubule-kinetochore attachments adopted in vivo.

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**In Vitro Studies of the Spindle Assembly Checkpoint in Yeast**

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Although extremely rare, errors in chromosome segregation lead to aneuploidy, which has been linked to cancer and birth defects. Dividing cells rely on a surveillance mechanism called the spindle assembly checkpoint (SAC), which ensures that chromosomes are properly segregated during mitosis. The SAC, comprising several proteins on the mitotic kinetochore emits a diffusible 'wait' signal upon detection of improper and/or unattached kinetochores, delaying mitosis. Proper attachment and biorientation silences the 'wait' signal allowing progress through mitosis. 'Wait' signal silencing is thought of in two possible ways: First, the SAC responds to kinetochore-microtubule tension. Second, the SAC responds to attachment of microtubules to kinetochores, independent of tension. This attachment-tension debate is still unresolved due to the challenge of independently controlling attachment states and levels of tension on kinetochores in vivo. To help shed more light, we purified mitotic kinetochores from budding yeast with core kinetochore components, Mtw1p and Nuf2p, fluorescently labeled. We immobilized purified kinetochores onto a coverslip in our in vitro assay, and using a very sensitive Total Internal